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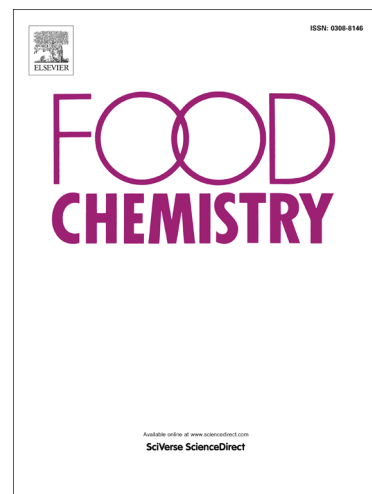
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**Effect of pulsed light treatments on quality and antioxidant properties of fresh-cut strawberries**

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**ABSTRACT**

Fresh-cut strawberries were subjected to pulsed light treatments (4, 8, 12 and 16 J cm<sup>-2</sup>) combined with a stabilizing dip. Quality changes and antioxidant properties were evaluated for 14 days at 5 °C. The treatments delayed fungal for the studied period. Surface color of treated fresh-cut strawberries was preserved in both internal and external surfaces. Doses of 4 and 8 J cm<sup>-2</sup> reduced softening incidence over storage. No significant differences between the total phenolic contents of untreated and treated fresh-cut strawberries were observed during storage. Vitamin C and total anthocyanin contents of the samples treated at low energy doses were maintained, whereas those of slices treated at the highest energy dose decreased between 20 and 30%, respectively. On the other hand, initial antioxidant capacity was better kept in all samples during storage regardless the applied dose. Hence, 4 and 8 J cm<sup>-2</sup> were the most effective treatments for maintaining quality and antioxidant properties of fresh-cut strawberries.

*Industrial relevance:*

Fresh-cut strawberries may be sold as a highly convenient, healthy and fully edible product. However, processing and packaging conditions need to ensure the maintenance of their quality characteristics and content of bioactive compounds. This study provides scientific evidence regarding the beneficial effects of pulsed light treatments combined with a quality-stabilizing dip for extending the shelf-life of fresh-cut fruit. These combined treatments may be economically viable at industrial level because of their low energy requirements and reduced treatment time.

*Keywords:* Pulsed light; Fresh-cut strawberry; Antioxidant properties

## 1. Introduction

Strawberry consumption has been widely associated with the prevention of several chronic pathologies, and the slowdown of the progression of aging (Giampieri *et al.*, 2014, 2017 & 2018). On the other hand, there is a growing demand for both healthy and convenient food products that has triggered the development of a market for fresh-cut fruit commodities. However, despite the health benefits of strawberry, which are related to the high content in vitamin C and phenolic compounds, mainly anthocyanins, the market sale of fresh-cut strawberries is not yet consolidated. High susceptibility to spoilage, softening, water loss, and browning are key aspects that jeopardize their industrial development. Refrigeration and, to a lesser extent, modified atmosphere packaging, are key technologies that allow shelf-life extension by reducing microbial growth and physicochemical decay. In addition, some other treatments are often used in order to control decay and physiological disorders without affecting quality of fresh-cut produce. Ascorbic acid (AA) dips are commercially used to delay browning and discoloration of fresh-cut products. Furthermore, Chen *et al.* (2011), García, Herrera, & Morilla (1996), and Lara, García, & Vendrell (2004) reported the action of calcium salts in preventing decay and firmness loss of strawberry fruits. Nevertheless, the beneficial effects of these treatments are limited and do not allow counteracting the deleterious impact of processing and handling operations on the shelf-life of fresh-cut strawberries. Consequently, other supplementary treatments are investigated to

further extend the shelf-life of the product over storage (Alexandre, Brandão, & Silva, 2012).

Pulsed light (PL) is an emerging decontamination technology that has been approved by the U.S. Food and Drug Administration (FDA, 2015) for the treatment of foods at a maximum dose of  $12 \text{ J cm}^{-2}$ . This technology is based on the use of intense broad spectrum light for short periods. Even though the UV-light portion of the spectrum has been identified as the main responsible for the antimicrobial effect of the treatment, other side effects have been related to visible and infrared wavelength ranges. The mechanisms underlying microbial inactivation by PL have been associated to the damage caused by photochemical changes in the genetic material and by localized heating (Demirci, & Krishnamurthy, 2011). PL-treatments may cause inactivation of bacteria, fungi, and viruses in shorter times than those required by continuous UV light (Soliva-Fortuny, Elez-Martínez, & Martín-Belloso, 2014). PL-treatments have been suggested as an eco-friendly technology to control decay and maintain the quality of fresh strawberry fruits (Bialka & Demirci, 2008; Bialka, Demirci, & Puri, 2008; Duarte-Molina, Gómez, Castro, & Alzamora, 2016; Luksiene, Buchovec, & Viskelis, 2013). Marquenie, Michiels, Van Impe, Schrevers, & Nicolaï (2003) used light pulses alone or in combination with thermal or UV-C treatments for surface decontamination of strawberry fruit, and reported that PL-treatments alone did not delay fungal development during storage. On the other hand, Luksiene et al. (2013) studied the impact of PL on antioxidant properties only immediately after application, and found no significant differences between the values of treated and untreated strawberries. However, the influence of PL-treatments on fresh-cut strawberry has not been yet evaluated. Although several studies have demonstrated the impact of PL on antioxidant properties of fresh-cut fruit and vegetables, such as apples, mangoes, cantaloupes, and mushrooms

(Avalos Llano, Marsellés-Fontanet, Martín-Belloso & Soliva-Fortuny, 2016; Charles, Vidal, Olive, Filgueiras, & Sallanon, 2013; Koh, Noranizan, Karim, & Hanani, 2016; Oms-Oliu, Aguiló-Aguayo, Martín-Belloso, & Soliva-Fortuny, 2010), no information is available regarding the use of this technology for preserving antioxidant quality of fresh-cut strawberries during storage. Therefore, the aim of the present work was to study the effect of PL-treatments combined with an ascorbic acid – calcium lactate (AA–CaL) dip on the quality and antioxidant properties of fresh-cut strawberries over refrigerated storage.

## 2. Materials and Methods

### 2.1. Fruit Processing and packaging

Strawberries (*Fragaria ananassa* Duch, cv. Camarosa) were purchased in a local supplier in Lleida (Spain). Fruits showing 90% red surface color, uniform size and absence of external damages were selected. Strawberries were washed and sanitized by immersion into a 200  $\mu\text{L L}^{-1}$  sodium hypochlorite solution for 1 min, then rinsed, and dried with paper cloth prior to cutting. The calyxes were removed, and the fruits were sliced longitudinally into four slices (quarters) with a sharp knife. Strawberry slices were dipped into an AA–calcium lactate (CaL) solution (10 °C, 1 min) in a solid/liquid ratio of 1:2. The solution contained 10 g  $\text{L}^{-1}$  L-ascorbic acid and 35.3 g  $\text{L}^{-1}$  pentahydrated calcium lactate. Once the excess of solution was allowed to drain for 5 min, eight slices with a weight of approximately 7 g each (ca. 60 g) were placed separately in transparent polypropylene trays. The packages were thermosealed using a packaging machine (ILPRA Food Pack Basic V/G, ILPRA Systems, Vigevano, PV, Italy). A sealing film with thickness of 64  $\mu\text{m}$  and permeability to oxygen of 110  $\text{cm}^3 \text{O}_2 \text{m}^{-2} \text{bar}^{-1} \text{day}^{-1}$  at 23 °C and 0% RH (Tecnopack

SRL, Mortara, Italy) was used to minimize water loss. The film transmittance was 85% for the wavelengths between the 200-320 nm range, over 97% for the overall incident UV-radiation (200-400 nm) and almost 100% of the visible-infrared radiation (400-1100 nm). Hence, strawberry slices were subjected to the PL-treatments inside of the packages once these had already been sealed. Untreated slices, either immersed or not into the AA–CaL dip, were also prepared to be used as a reference.

## *2.2. Pulsed light treatments and storage*

A pulsed UV system Model XeMaticA-2 L (360° sample illumination) (SteriBeam Systems GmbH, Kehl, Germany) was used to apply the PL-treatments. The equipment consists of two air cooled Xenon lamps situated 8.5 cm far above and below a quartz sample shelf. The emitted spectrum wavelengths range from 180 to 1100 nm with 15–20% of the light in the UV region. Each pulse lasts 0.3 ms and the energy deposition delivered by each lamp at the sample level was set at  $0.4 \text{ J}\cdot\text{cm}^{-2}$  per pulse. Each package was individually treated with 10, 20, 30 or 40 pulses, corresponding to doses of 4, 8, 12, and  $16 \text{ J}\cdot\text{cm}^{-2}$  per side, respectively. The amount of energy received by a photodiode detector placed at the sample holder was measured to calculate the PL-doses. The photodiode was connected to an oscilloscope and the recorded signal was transformed into radiance values using a calibration source equipped with a standard light as per the instruction of the manufacturer. In addition, a Makrolon® filter was used to evaluate the amount of radiation in the UV range. Broad-range and UV-range radiations emitted by top and bottom lamps were not much differentially blocked by the packaging materials. Photodiode readings revealed differences of less than 5% in the fluence at the sample level after passing through the package foils. In concomitance with the dose increase, the temperature may gradually rise



on the treated surfaces as well as inside the treatment chamber. Thus, measurements of the temperature at the sample shelf level over the most intense PL-treatment ( $16 \text{ J cm}^{-2}$ ) with a Testo thermometer (Testo, Cabrils, Spain) equipped with a type K thermocouple were carried out. The maximum temperature recorded was  $42.4 \pm 1.0 \text{ }^{\circ}\text{C}$  just after the most intense PL-treatment ( $16 \text{ J cm}^{-2}$ ). Finally, the trays were stored for 14 days at  $5 \pm 1 \text{ }^{\circ}\text{C}$  in darkness. Ten PL-treatment replicates were independently carried out for each evaluated condition and replicate trays were randomly withdrawn at 0, 3, 6, 10 and 14 days for analysis. Fresh slices were used for quality determinations, whereas a portion of 25 g was immediately freeze-dried and stored at  $-40 \text{ }^{\circ}\text{C}$  until extraction and determination of antioxidant compounds.

### *2.3. Fungal decay incidence*

Fungal decay incidence was determined according to Duarte-Molina et al. (2016), and Jeong, Chu, Lee, Cho, & Park (2016), and expressed as the number of slices affected by macroscopic fungal development with respect to the total number of evaluated slices. Two replicate packages were randomly withdrawn and 16 slices in total were visually evaluated for each treatment and sampling time.

### *2.4. Browning incidence, overall quality index, and surface color*

Browning incidence was evaluated according to Lentheric, Pintó, Graell, & Larrigaudiere (2003). Result was expressed as the number of slices affected by surface browning with respect to the total number of evaluated slices. Two replicate packages were randomly withdrawn and 16 slices in total were visually evaluated for each treatment and sampling time.

Overall visual quality was examined under white light according to the method described by Mahmoud, Bhagat, & Linton (2007) with modifications. Briefly, the general appearance was evaluated taking into account browning, shriveling, and the presence of macroscopic fungal development on individual strawberry slices. Scores from 1 to 9 (1: extremely bad, 2: very bad, 3: moderately bad, 4: slightly bad, 5: acceptable, 6: slightly good, 7: moderately good, 8: very good, 9: extremely good) were used to calculate an overall quality index,  $OQI = \sum (\text{score} \times n) / N$ , where  $n$  is the number of slices corresponding to each score, and  $N$  is the total number of analyzed slices. Scores lower than 5 were considered to be indicative that samples lost marketable quality. Two replicate packages were randomly withdrawn and 16 slices in total were visually evaluated for each treatment and sampling time.

Surface color values were directly measured with a colorimeter (Chroma Meter Model CR-400, Konica Minolta Sensing Inc., Osaka, Japan) using a D65 illuminant and an observation angle of  $10^\circ$ . The instrument was calibrated using a standard white reflector plate. The color parameters were measured on both internal (cut) and external surfaces. In the cut surface, the measurements were made on opposite sides of the slice, right and left. Three readings of  $L^*$  (lightness),  $a^*$  (green-red chromaticity) and  $b^*$  (blue-yellow chromaticity) coordinates were recorded from each zone of the slice for calculating hue angle  $H = \tan^{-1}(b^*/a^*)$ , chroma value  $C = (a^{*2} + b^{*2})^{1/2}$ . The parameters of the internal zone were expressed as the mean of the readings on the two opposite sides. During storage, two replicate packages were randomly withdrawn and 16 slices in total were evaluated for each treatment and sampling time.

## 2.5. Firmness

Firmness was evaluated with a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, U.K.) by measuring the force required for a 4-mm-dia probe to penetrate pieces of slices of 10 mm height to a depth of 5 mm at a rate of 5 mm s<sup>-1</sup> and automatic return. Samples were taken by cutting transversally into 10 mm height piece from the central zone of each slice, and were placed perpendicular to the probe in order to be penetrated in their geometric center. Two replicate packages were randomly withdrawn and 16 slices in total were evaluated for each treatment and storage time. Results were expressed as the maximum penetration force in N.

## 2.6. Antioxidant properties

### 2.6.1. Preparation of methanolic extracts

Freeze-dried samples of 15 g were mixed with 30 mL of 80% methanol using an Ultra-Turrax homogenizer (IKA Model T 25 Digital, Germany) (2 min, 10,200 rpm, 0 °C). The mixture was centrifuged at 12,500 rpm for 15 min at 4 °C (AVANTI J-26 XP centrifuge, Beckman Coulter Inc., Fullerton, CA, USA). The resulting solution constituted the methanolic extract.

### 2.6.2. Determination of total phenolic compounds via Folin-Ciocalteu, and Fast Blue BB methods

The total phenolic content was determined according to the Folin-Ciocalteu procedure (Singleton, Orthofer, & Lamuela-Raventós, 1999). To that purpose 0.25 mL of methanolic extract was added to 10 mL of water and 0.25 mL of Folin-Ciocalteu reagent (Sigma Chemical, St. Louis, MO, USA). After 1 min, 8 mL of 7.5% w/v Na<sub>2</sub>CO<sub>3</sub> was added. The

absorbance at 760 nm was measured after incubation at room temperature for 60 min in darkness conditions. Concentrations were calculated using gallic acid (Sigma Chemical, St. Louis, MO, USA) as standard in the 0–9  $\mu\text{g mL}^{-1}$  concentration range. Results were expressed as gallic acid equivalents on a fresh weight basis.

Additionally, the total phenolic content was also determined by using the Fast Blue BB [4-benzoylamino-2,5-dimethoxybenzenediazonium chloride hemi(-zinc chloride) salt] reagent (Medina, 2011). Namely, 0.4 mL of 0.1% w/v Fast Blue BB reagent was added to 4 mL of methanolic extract diluted 1:20, and this mixture was shaken for 30 s. Then, 0.4 mL of 5% w/v NaOH was added, and the resulting mixture was incubated for 90 min at room temperature in darkness conditions. The absorbance was measured at 420 nm. The total phenolic content was calculated using gallic acid dilutions in the 0–500  $\mu\text{g mL}^{-1}$  concentration range. Results were expressed as gallic acid equivalents on a fresh weight basis.

### 2.6.3. Total Anthocyanins

The total anthocyanin content was determined by using the pH differential method reported by Meyers, Watkins, Pritts, & Liu (2003). Aliquots of the methanolic extract were simultaneously diluted (1:10) by using the buffers 0.025 mol L<sup>-1</sup> potassium chloride (pH = 1) and 0.4 mol L<sup>-1</sup> sodium acetate (pH = 4.5), respectively. The absorbance values of the extracts at both pH = 1 and pH = 4.5 were then measured at 510 and 700 nm. The total anthocyanin content (TA) was calculated as:  $TA = [(A_{510\text{ nm}} - A_{700\text{ nm}})_{\text{pH}1.0} - (A_{510\text{ nm}} - A_{700\text{ nm}})_{\text{pH}4.5}] * MW * TV * DF * 1000 / (\epsilon * L * SW)$ , where A is the absorbance, MW is the molecular weight of pelargonidin-3-glucoside (433 g mol<sup>-1</sup>), TV is the total volume of the

methanolic extract (30 mL), DF is the dilution factor,  $\epsilon$  is the extinction coefficient (22,400 L mol<sup>-1</sup> cm<sup>-1</sup>), L is the cuvette length (1 cm), and SW is the sample weight (15 g). Results were expressed as pelargonidin-3-glucoside equivalents on a fresh weight basis.

#### 2.6.4. Vitamin C

Vitamin C content was analyzed by HPLC according to the extraction procedure and chromatographic conditions described by Odriozola-Serrano, Hernández-Jover, & Martín-Belloso (2007). Freeze-dried samples of 0.1 g were extracted twice with 5 mL of a solution containing 45 g L<sup>-1</sup> metaphosphoric acid and 7.2 g L<sup>-1</sup> DL-1,4-dithiotreitol, using an Ultra-Turrax (IKA Model T 25 Digital, Germany) homogenizer (2 min, 10,200 rpm, 0 °C). The mixture was centrifuged at 6,000 rpm for 15 min (Hettich ZENTRIFUGEN EBA 21, Andreas Hettich GmbH & co. KG, Tuttlingen, Germany). The supernatant was recovered, made up to 10 mL, and then passed through a Millipore 0.45 µm membrane. An aliquot of 20 µL was injected into the HPLC system using a reverse-phase C18 Spherisorb® ODS2 (5 mm) stainless steel column (250 mm × 4.6 mm). The mobile phase was a 0.01% v/v sulphuric acid solution adjusted to pH = 2.6. The flow rate was fixed at 1.0 mL/min at room temperature. Detection was performed with a 2996 Photodiode Array Detector (Waters, Milford, MA) set at 245 nm. Vitamin C content was calculated using ascorbic acid (Scharlau Chemie S.A., Barcelona, Spain) as standard in the 0-15 µg mL<sup>-1</sup> concentration range. Results were expressed as mg of vitamin C on a fresh weight basis.

#### 2.6.5. Antioxidant capacity

The antioxidant capacity was determined by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>) radical (Fluka Chemie, Switzerland) according to de Ancos, Sgroppo, Plaza, & Cano (2002). Aliquots of 0.01 mL of the methanolic extract were mixed with 3.9 mL of a 0.025 g L<sup>-1</sup> methanolic DPPH<sup>•</sup> solution and 0.09 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. The absorbance was measured with a CECIL CE 1021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) at 517 nm against a blank of methanol without DPPH<sup>•</sup>. The inhibition of the DPPH<sup>•</sup> radical was calculated and plotted as a function of the concentration of Trolox for the standard reference data. The final DPPH<sup>•</sup> value was calculated using a calibration curve. Results were expressed as Trolox equivalents on a fresh weight basis.

### *2.7. Statistical analysis*

Statistical analysis was performed using the InfoStat v. 2015I software package (Di Rienzo, Casanoves, Balzarini, Gonzalez, Tablada, & Robledo, 2015). Three independent experiments were performed. Means and standard deviation of the three experiments were depicted. Data were analyzed by means of an analysis of variance, and the means were compared using the Duncan's test at  $p < 0.05$ .

## **3. Results and discussion**

### *3.1. Fungal decay incidence*

Symptoms of fungal decay were not evident in PL-treated cut strawberries immersed in the AA-CaL dip through 10 days at 5 °C, regardless the dose applied (Fig. 1a). At the end of storage, the decay incidences of PL-treated slices dipped into the AA-CaL solution were

lower than those of slices only dipped into AA-CaL solution and those of untreated strawberry slices. PL-treatments have been reported to cause damage to cells by induction of photochemical changes in DNA/RNA components and/or by localized heating (Demirci & Krisnamurthy, 2011). In the present study, the germicidal effect of the absorbed UV-light energy was presumably behind microbial inactivation, as treatment temperatures did not exceed 42 °C even for the most intense doses (16 J·cm<sup>-2</sup>).

Duarte-Molina et al. (2016) also reported that PL-treatments reduced the fungal incidence on whole strawberry fruits. However, the application of PL-treatments to fresh-cut strawberries in the present study allowed inhibiting fungal development for a longer period. This delay of fungal decay may be related to a lower presence of achenes on the fresh-cut surfaces to be treated, which in the fresh whole fruit may have a protective shadowing effect against pulsed UV-light (Bialka, & Demirci, 2008; Duarte-Molina et al., 2016).

### *3.2. Browning incidence, overall quality index, and surface color*

Neither slices only dipped into AA-CaL solution nor those PL-treated and immersed in the AA-CaL dip developed signs of browning for 10 days of storage (Fig. 1b). Hence, the AA-CaL solution effectively prevented browning regardless the PL dose applied. In contrast, more than a 50% of untreated slices developed evident signs of browning over storage. Gómez, García-Loredo, Nieto, Salvatori, Guerrero, & Alzamora (2012) and Oms-Oliu et al. (2010) reported that AA in combination with PL-treatments prevented the browning in fresh-cut apples and mushrooms, although dose-dependent effects were reported. The authors suggested that high PL-doses triggered enzymatic browning development, which may be attributed to thermal damage triggering the decompartmentation of enzymes and their substrates.

Browning and fungal development were the most fundamental factors affecting the appearance of fresh-cut strawberries (Fig. 1). As a consequence of the reduced incidence of decay and browning, treated slices exhibited higher overall quality index values than untreated slices (Fig. 1c). Namely, overall quality index of PL-treated fresh-cut strawberries dipped into the AA-CaL solution ranged between 6.13 and 7, whereas the score for fresh-cut strawberries only dipped into AA-CaL solution was  $5.39 \pm 0.15$  after 14 days at 5 °C. In contrast, overall quality index of untreated slices dramatically decreased through storage to values of  $1.67 \pm 0.06$ . Luksiene et al. (2013) reported that PL-treated whole strawberries subjected to  $5.4 \text{ J cm}^{-2}$  better maintained their overall visual quality than untreated fruits for up to 4 days at 6 °C.

Color was measured on both internal (cut) and external surfaces. Hue angle values of cut surfaces slightly decreased, whereas those of external zones remained unchanged regardless the treatment applied over the analyzed storage period (Fig. 2a and c). As well, hue values of the external surface of treated fresh-cut strawberries were markedly higher than those of untreated slices. As shown in Fig. 2b and d, saturation or intensity (chroma value) of cut and external surfaces noticeably decreased during storage. However, the decrease of chroma values in strawberry pieces immersed in the AA-CaL dip, either PL-treated or not, was lower than in undipped pieces.

Although the internal cut surfaces of fresh-cut strawberries dipped into AA-CaL solution showed less red color intensity at the end of storage,  $L^*$  and  $a^*$  values did not change over storage regardless the applied PL treatment (Fig. 2, 3). However, a marked decrease in chroma and  $a^*$  values (Fig. 2, 3) was observed in cut zones of untreated slices, probably as a consequence of browning and discoloration phenomena occurring in the pith (Fig. S1a). Interestingly, no signs of browning were evidenced in most of the slices subjected to the



highest applied dose ( $16 \text{ J}\cdot\text{cm}^{-2}$ ) after 14 days of storage (Fig. 1b and S1b) as the AA-CaL dip prevented the development of browning.

As Fig. 3c shows, the lightness parameter ( $L^*$ ) of external zones was maintained in all samples throughout storage. On the other hand,  $a^*$  parameter of external zones decreased in dependence on the treatment applied (Fig. 3d). A slight  $a^*$  decrease was detected in fresh-cut strawberries treated at low doses, whereas more marked decrease was found in slices treated at  $16 \text{ J}\cdot\text{cm}^{-2}$  that may be related to the changes in anthocyanin content reported below.

### 3.3. Firmness

No significant firmness changes were found in fresh-cut strawberries immediately after the application of PL-treatments (Fig. 1d), as previously reported by Duarte-Molina et al. (2016) and Luksiene et al. (2013) in whole strawberries. Although the immersion into a calcium lactate solution did not result into significantly firmer fruit, the combination with 4 and  $8 \text{ J}\cdot\text{cm}^{-2}$  doses best kept the initial firmness values during storage. Similarly, Ramos-Villarroel, Martín-Belloso, & Soliva-Fortuny (2011) reported that calcium lactate combined with a PL-treatment effectively prevented softening in fresh-cut avocado. The reduction of softening may be explained by the stress caused by PL treatment, which is likely to induce cell wall strengthening and a major loss of integrity of the walls of hypodermis cells, as reported by Duarte-Molina et al. (2016) in whole strawberries observed via light and transmission electron microscopy. This improved maintenance of tissue integrity may be associated with a decrease in the biological activity of enzymes and proteins involved in cell wall degradation. Direct exposure to UV-C wavelengths has been reported to cause a decrease in the transcription of genes encoding these enzymes and proteins (Pan, Vicente,

Martínez, Chaves, & Civello, 2004; Pombo, Dotto, Martínez, & Civello, 2009). However, PL-treated fresh-cut strawberries subjected to 12 and 16 J·cm<sup>-2</sup> and immersed in the AA-CaL dip showed similar firmness decreases than untreated slices and those only dipped into AA-CaL solution. Other authors have reported a firmness decrease in PL-treated and untreated whole strawberry fruits during storage (Marquenie et al., 2003). The application of the greatest PL-doses likely caused severe injury to the irradiated tissues, which was suggested by Duarte-Molina et al. (2016) to explain firmness loss.

### *3.4. Antioxidant properties*

#### *3.4.1. Total phenolic compounds*

During storage, no effects of PL-treatments on the total phenolic content were observed (Fig. 4a, and b). Total phenolic compounds determined via both methods, Folin–Ciocalteu (FC) and Fast Blue BB (FBBB) remained without substantial changes in untreated and treated fresh-cut strawberries at all studied doses. No significant differences between samples subjected to different processing conditions were observed. On the other hand, phenolic concentrations over storage obtained via the FBBB test were 18-21% higher than those obtained via the FC method, in accordance with the results previously obtained for whole strawberry (Lester, Lewers, Medina, & Saftner, 2012; Medina, 2011). Total phenolic compounds measured with the Folin–Ciocalteu (FC) reagent should be interpreted as a measurement of the total reducing capacity because of the interferences produced by non-phenolic substances, such as ascorbic acid (AA), glucose, fructose, sulphites, tyrosine, tryptophan, and proteins containing these amino acids with the FC reagent (Lester et al., 2012; Medina, 2011; Prior, Wu, & Schaich, 2005; Proteggente et al., 2002). In our work,

the interference was most likely caused by the high amounts of AA added with the stabilizing dip. Therefore, the use of the Fast Blue BB (FBBB) method for the determination of total phenolics in fresh-cut commodities is suggested to avoid the interferences with other reducing substances, thus allowing a better estimation of the total phenolic content.

### 3.4.2. Total anthocyanins

No significant differences in total anthocyanin contents were found immediately after PL-treatments (Figure 5a), as reported by Luksiene et al. (2013) in whole strawberries. During storage, anthocyanin content slightly decreased in slices only dipped into AA-CaL solution that may be due to the presence of AA, which speeds up the degradation of anthocyanins in strawberry (Garzón & Wrolstad, 2002). However, fresh-cut strawberries treated with PL-doses of 4, 8 and 12 J·cm<sup>-2</sup> and immersed in the AA-CaL dip maintained the total anthocyanins content without significant changes over storage. Although, anthocyanin content slightly decreased in slices treated with PL-dose of 16 J·cm<sup>-2</sup> and immersed in the AA-CaL dip, these samples retained about 80% initial anthocyanins content at the end of storage.

Berries and berry products owe their attractive color to their high content of anthocyanins. Several authors have studied the effects of storage and/or preservative treatments on the stability of color and anthocyanins (Busso Casati, Baeza, & Sánchez, 2017; Howard, Brownmiller, Mauromoustakos, & Prior, 2016; Segantini, Threlfall, Clark, Brownmiller, Howard, & Lawless, 2017). In our work, anthocyanin variation may explain changes in chroma and a\* values. Nevertheless, an anthocyanin decrease was only found in slices only dipped into AA-CaL solution and those treated at 16 J·cm<sup>-2</sup> and immersed in the AA-CaL

dip (Fig. 2, 3, and 5). The low doses allowed avoiding the deterioration of color and the anthocyanins loss.

#### 3.4.3. Vitamin C

As shown in Fig. 5b, those slices only dipped into the AA-CaL solution exhibited 23-28% higher vitamin C content than untreated slices for 6 days of storage. Immediately after PL-treatments, no differences in the vitamin C content was found between slices flashed with doses lower than  $16 \text{ J cm}^{-2}$ , which is consistent with the findings of Luksiene et al. (2013) for whole strawberries. On the other hand, the vitamin C content of fresh-cut strawberries treated at  $4 \text{ J cm}^{-2}$  was maintained throughout storage, whereas that of slices treated at  $8 \text{ J cm}^{-2}$  was kept for 6 days, and then slightly decreased. In slices treated at 12 and  $16 \text{ J cm}^{-2}$ , vitamin C values underwent a substantial depletion throughout storage. This decrease may be due to photochemical effects rather than to thermal effects, as temperature readings never exceeded  $42^\circ\text{C}$ . On the other hand, the higher reduction in slices subjected to the greatest PL-doses may be evidence signs of oxidative injury in the tissues irradiated with those doses, as AA is highly prone to oxidation upon wounding (Andrade Cuvi, Vicente, Concellón, & Chaves, 2011).

#### 3.4.4. Antioxidant capacity

Several methods have been used to measure antioxidant capacity and each one provides partial information. However not all methods have a direct relevance to *in vivo* antioxidant status. Although using different radical/oxidant sources may be helpful in determining antioxidant capacity and understanding health benefits of different berries, berry products,

and foods (Morita, Naito, Yoshikawa, & Niki, 2017; and Prior, Sintara, & Chang, 2016) only the DPPH· test was used in the present study.

As Fig. 4c shows, the initial antioxidant capacity was retained in all samples during storage. In addition, slices only dipped into AA-CaL solution and those PL-treated and immersed in the AA-CaL dip showed slightly higher antioxidant capacity values than untreated slices due to the presence of AA in the composition of the stabilizing solution. On the other hand, PL-treatments did not affect the antioxidant capacity throughout storage.

The presence of vitamin C, flavonoids, and phenolic acids contribute to the overall antioxidant capacity of strawberries. Thus, changes in antioxidant capacity should be expected to be associated with the changes in these compounds during storage. A principal component analysis was used to reveal the interrelationships between the different variables (antioxidant capacity, total phenolics via FC and FBBB, vitamin C, and total anthocyanins). As shown in Fig. S2, the sum of the variances explained by the first two components, principal component 1 and principal component 2, was 75%. Principal component 1 (PC 1) accounted for 52.5% of the overall variance, whereas principal component 2 (PC 2) was responsible for 22.8% of the variance. The variables antioxidant capacity and total phenolics via FBBB did not correlate well. However, positive correlations between the variables antioxidant capacity, total phenolics via FC, vitamin C, and total anthocyanins could be drawn. Total phenolics via FC and vitamin C were more strongly correlated to antioxidant capacity than total anthocyanins. In addition, total anthocyanins were similarly correlated to total phenolics via FC and FBBB as anthocyanins are quantified by both methods. On the other hand, vitamin C was better correlated to total phenolics via FC than to total phenolics FBBB, which is consistent with the already reported interference of ascorbic acid with the FC reagent. Odriozola-Serrano et al. (2010) reported that the

antioxidant activity of fresh-cut strawberries stored under MAP conditions was highly related to vitamin C levels. On the other hand, Erkan, Wang, & Wang (2008) found that the ORAC values did not correlate well with anthocyanin contents in strawberry fruits treated with continuous UV-C. As well, Tulipani, Marzban, Herndl, Laimer, Mezzetti, & Battino (2011) and Tulipani et al. (2008) reported that the total antioxidant capacity was not strongly correlated with the anthocyanin content in different strawberry genotypes. In our work, the results suggest that the antioxidant capacity trend was associated with the evolution of vitamin C rather than to non-anthocyanin phenolic compounds, which may be due to the presence of vitamin C and anthocyanins as the major antioxidant components in strawberries (Hannum, 2004).

#### **4. Conclusions**

Pulsed light treatments combined with the AA-CaL dip affected positively the overall quality of fresh-cut strawberries by reducing and/or inhibiting fungal development and browning during cold storage. In addition, low energy doses reduced softening incidence over storage. On the other hand, no dramatic effects on antioxidant properties were found. Although the PL-treatment at  $8 \text{ J cm}^{-2}$  retained 90% of the initial content of vitamin C, the other studied properties were maintained. Thus, PL-doses of 4 and  $8 \text{ J cm}^{-2}$  in combination with the AA-CaL dip may be useful non-thermal strategy to extend the shelf life and maintain the antioxidant quality of fresh-cut strawberries.

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## Figure captions

**Fig. 1** Fungal decay (a), and browning (b) incidences, overall quality index (c), and firmness values (d) of slices exposed to light pulses (expressed as  $\text{J}\cdot\text{cm}^{-2}$ ) and stored for 14 days at 5 °C. Dip: 10 g  $\text{L}^{-1}$  L-ascorbic acid + 35.3 g  $\text{L}^{-1}$  pentahydrate calcium lactate. Data shown are mean of fourteen (a, b, and c) and twelve (d) repetitions from two replicate packages  $\pm$  standard deviation.

**Fig. 2** Hue angle (a, c), and chroma values (b, d) of internal and external zones, respectively, in slices exposed to light pulses (expressed as  $\text{J}\cdot\text{cm}^{-2}$ ) and stored for 14 days at 5 °C. Dip: 10 g  $\text{L}^{-1}$  L-ascorbic acid + 35.3 g  $\text{L}^{-1}$  pentahydrate calcium lactate. Data shown are mean of twelve repetitions from two replicate packages  $\pm$  standard deviation.

**Fig. 3**  $L^*$  (a, c), and  $a^*$  (b, d) values of internal and external zones, respectively, in slices exposed to light pulses (expressed as  $\text{J}\cdot\text{cm}^{-2}$ ) and stored for 14 days at 5 °C. Dip: 10 g  $\text{L}^{-1}$  L-ascorbic acid + 35.3 g  $\text{L}^{-1}$  pentahydrate calcium lactate. Data shown are mean of twelve repetitions from two replicate packages  $\pm$  standard deviation.

**Fig. 4** Total phenolics (a) and flavonoids (b) contents, and antioxidant capacity (c) of slices exposed to light pulses (expressed as  $\text{J}\cdot\text{cm}^{-2}$ ) and stored for 14 days at 5 °C. Dip: 10 g  $\text{L}^{-1}$  L-ascorbic acid + 35.3 g  $\text{L}^{-1}$  pentahydrate calcium lactate. Data shown are mean of two repetitions from two replicate packages  $\pm$  standard deviation.

**Fig. 5** Total anthocyanin (a) and vitamin C (b) contents of slices exposed to light pulses (expressed as  $\text{J}\cdot\text{cm}^{-2}$ ) and stored for 14 days at 5 °C. Dip: 10 g  $\text{L}^{-1}$  L-ascorbic acid + 35.3 g

$\text{L}^{-1}$  pentahydrate calcium lactate. Data shown are mean of two repetitions from two replicate packages  $\pm$  standard deviation.

### Supplementary material

Supplementary material can be found in the online version of this article:

**Fig. S1** General appearance of untreated sample (a) and strawberry slice with combined treatment ( $16 \text{ J}\cdot\text{cm}^{-2}$  + dip) (b) at 14 days of storage at  $5^\circ\text{C}$ . Dip:  $10 \text{ g L}^{-1}$  L-ascorbic acid +  $35.3 \text{ g L}^{-1}$  pentahydrate calcium lactate.

**Fig. S2** Comparison of fresh-cut strawberries stored 14 days of storage at  $5^\circ\text{C}$  and antioxidant properties (variables) using principal component analysis. Abbreviations of fresh-cut strawberries: U (untreated); D (dip), 4+D ( $4 \text{ J}\cdot\text{cm}^{-2}$  + dip); 8+D ( $8 \text{ J}\cdot\text{cm}^{-2}$  + dip); 12+D ( $12 \text{ J}\cdot\text{cm}^{-2}$  + dip); 16+D ( $16 \text{ J}\cdot\text{cm}^{-2}$  + dip). The numbers at the end of abbreviations indicate the storage day. Abbreviations of variables: A C (Antioxidant capacity), FC TP (Total phenolics via FC); Vit C (Vitamin C); T A (Total anthocyanins); FBBB TP (Total phenolics via FBBB).

Figure 1.

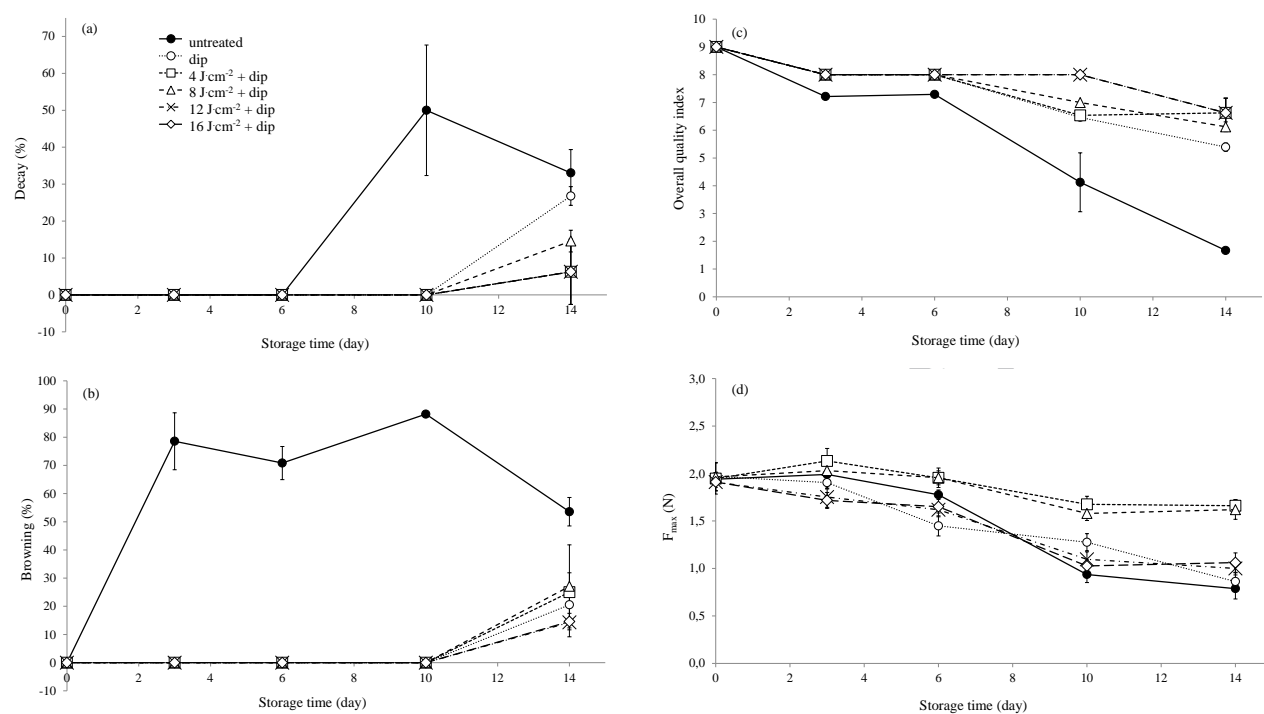


Figure 2.



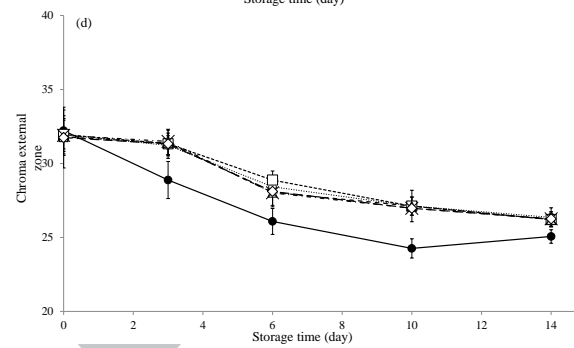
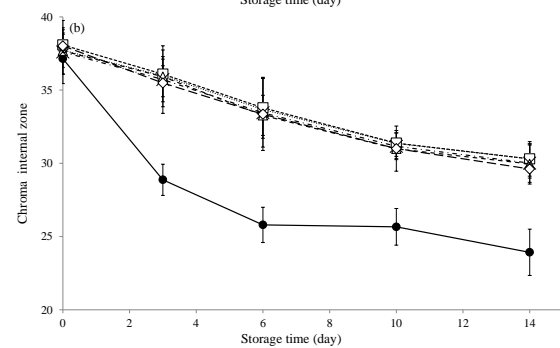
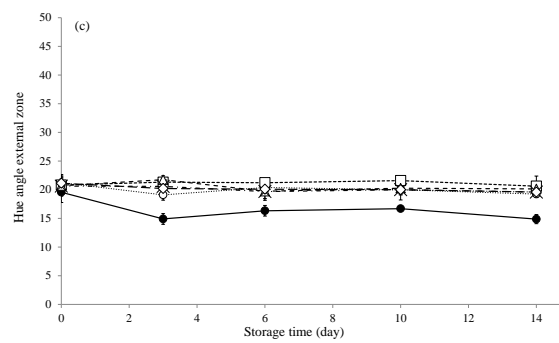
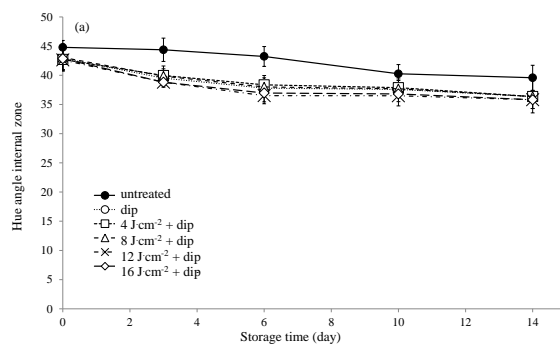


Figure 3.

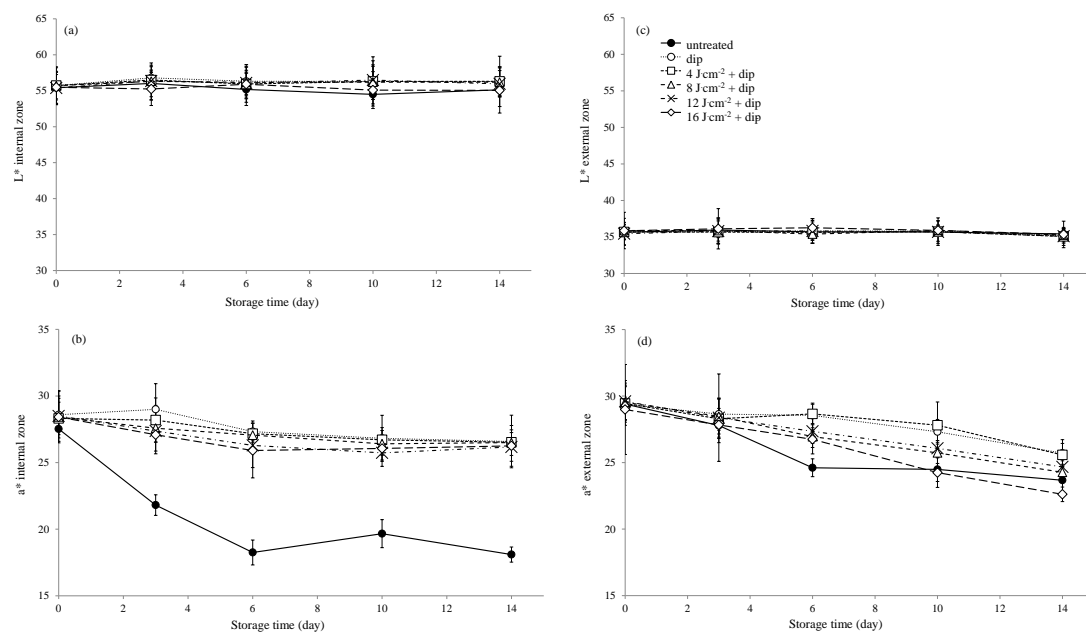


Figure 4.

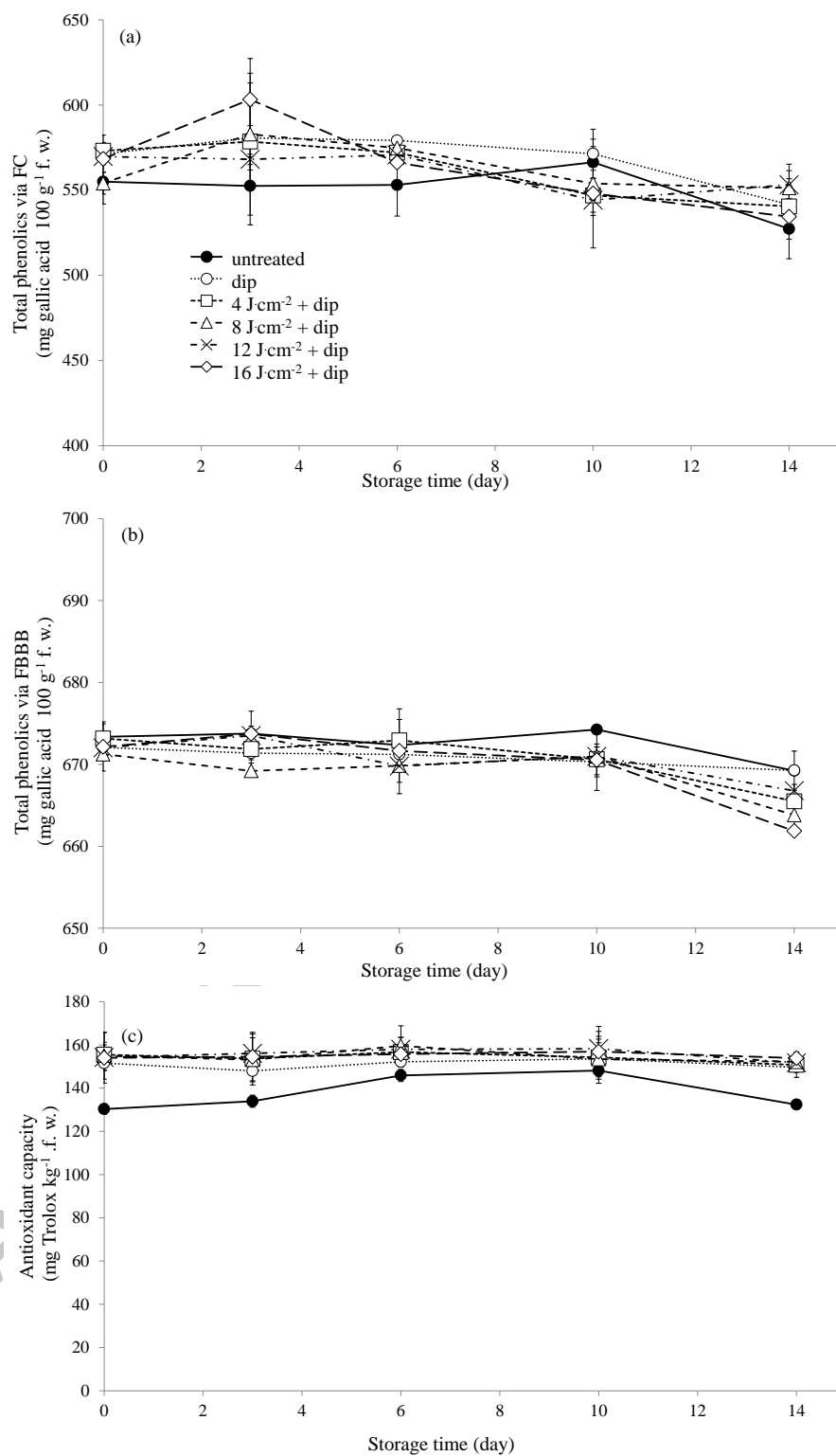
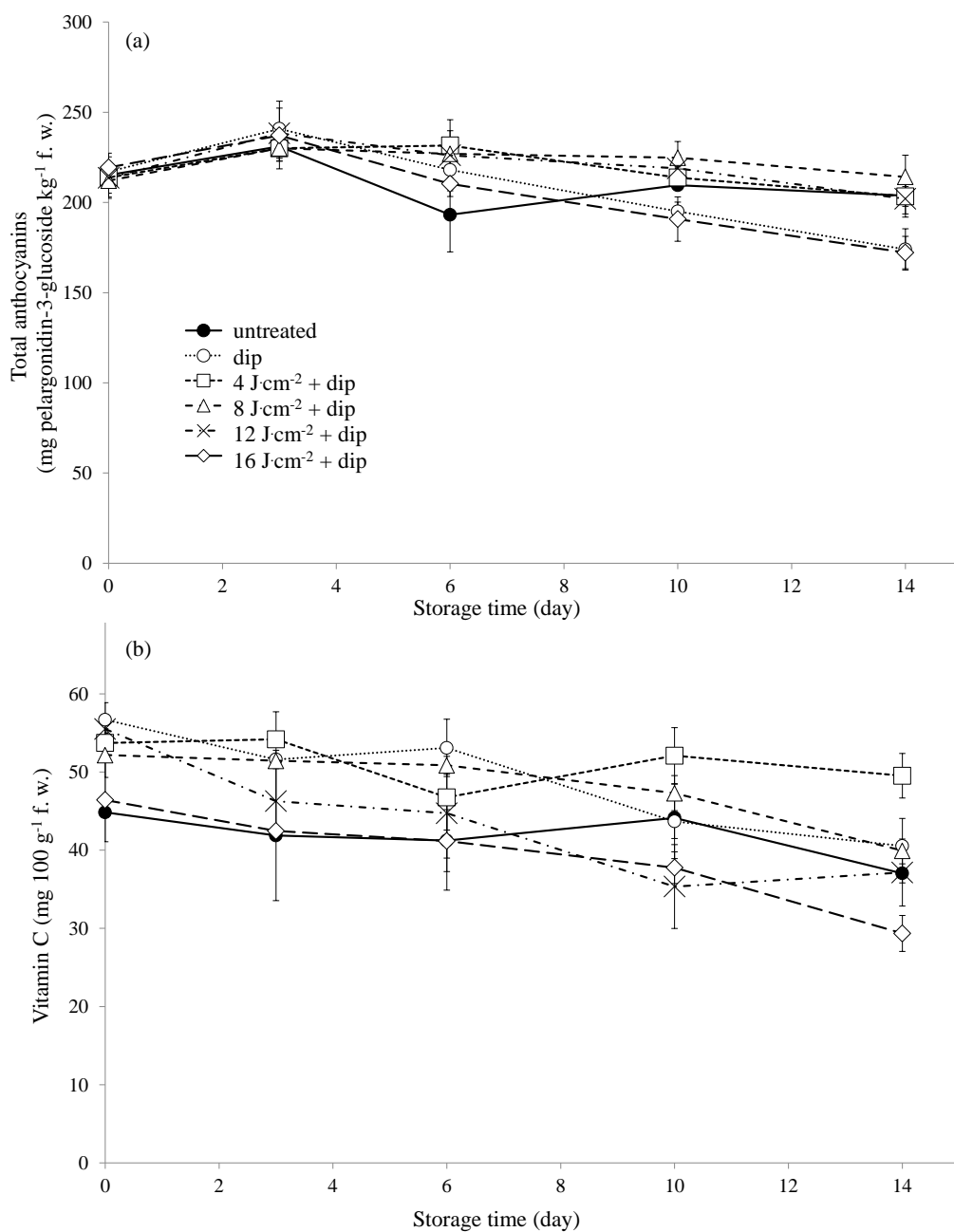


Figure 5.



**Highlights**

- Pulsed light (PL) treatments were applied for extending cut strawberry shelf life.
- PL-treatments were combined with an ascorbic acid/calcium lactate dip.
- Doses of 4 and 8 J cm<sup>-2</sup> helped to maintain quality and antioxidant characteristics.
- Fast Blue BB method estimated better total phenolics than Folin-Cicalteau method.
- Antioxidant capacity correlated positively to total vitamin C content.